



## CMV IgM EIA kit

(For *in vitro* diagnostic use only)

*Enzyme-linked immunosorbent assay for the detection of IgM antibody to Cytomegalovirus (CMV) infection*

### INTENDED USE

The CMV IgM kit is intended for use in the detection of IgM antibodies to Cytomegalovirus (CMV) infection.

### SUMMARY AND PRINCIPLE OF THE TEST

Cytomegalovirus is a herpes virus and a leading biological factor causing congenital abnormalities and complications among those who receive massive blood transfusions and immunosuppressive therapy. About half of pregnant women who contract a primary infection spread the disease to their fetus. When acquired in-utero, the infection may cause mental retardation, blindness, and/or deafness.

Serological tests for detecting the presence of antibody to CMV can provide valuable information regarding the history of previous infection, diagnosis of active or recent infection, as well as in screening blood for transfusions in newborns and immunocompromised recipients. ATLAS CMV IgM is an accurate serologic method to detect CMV IgM antibody for identification of CMV infection.

ATLAS CMV IgM Capture kit utilizes ELISA based on the antibody-capture technique. Patient sera are incubated with mouse monoclonal antibody against human IgM bound to the solid surface of a microtiter well. Patient IgM is 'captured' by the surface bound antibody. Unbound serum components are washed away. Patient anti-CMV IgM antibodies are 'detected' and bound by an immunocomplex, Enzyme conjugate, consisting of CMV antigen which is conjugated to horseradish peroxidase. Unbound conjugate is removed by aspiration and washing. Substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The absorbance of this end product can be read spectrophotometrically at 450 nm and is directly proportional to the concentration of IgM antibodies to CMV antigen present in the sample.

### REAGENTS

*Materials provided with the kits:*

1. 8X12 well microtiter strip: 1 plate, coated with anti-IgM.
2. Negative Control: 1 vial(0.2ml)
3. Positive Control: 1 vial (0.2ml)
4. Enzyme Conjugate: HRP-conjugated-CMV antigen(6 ml)
5. Wash Buffer: PBS, Tween. The buffer should be diluted with distilled water 1:20 before use.(40 ml)
6. Substrate Solution A: urea peroxide.(6 ml)
7. Substrate Solution B: TMB.(6 ml)
8. Stop Solution: 2N Sulfuric Acid(6 ml)

*Materials required but not provided:*

1. Micropipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled or deionized water.

4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader with 450nm wavelength
8. Timer

### PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Do not use kit beyond expiration date.
3. Do not mix components from kits with different lot number.
4. Avoid microbial contamination of reagents.
5. Do not pipette reagent by mouth and no smoking or eating while performing assays.
6. Wear gloves during the whole process and avoid reagents or specimen spilling-out.
7. Wipe up the spills using 5% hypochlorite solution.
8. Decontaminate all liquids or solid wastes before depositing.

### SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

### STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C. **DO NOT FREEZE KIT COMPONENTS.** The microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

### ASSAY PROCEDURE

1. Allow all components to reach room temperature before use.
2. Dispense 50 µl of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control, and 50µl of serum or plasma samples into respective test wells
3. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
4. Add one drop (50 µl) of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add Enzyme Conjugate to the blank well.
5. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
6. Wash each well 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
7. Add 50 µl of Substrate Solution A (HRP substrate) to each well, and then add 50 µl of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 min.
8. Add one drop (50 µl) of Stop Solution to each well to stop the color reaction. Read OD values of all samples at 450 nm.

### INTERPRETATION OF RESULTS

**EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):**

Cut Off: 0.10+Avarage OD value of Negative Control

Positive: OD value is equal to or greater than the Cut Off value

Negative: OD value is less than the Cut Off value

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

#### **LIMITATIONS OF THE ASSAY**

1. To prevent false negative and false positive IgM test results caused by the presence of specific IgG and rheumatoid factor (RF) in some specimens, reagents provided in this kit has been formulated to resolve these interferences. However, specimens with extremely high RF and high autoimmune antibodies, the possibility of these interferences cannot be ruled out entirely.
2. As with other serological tests, the results obtained with the CMV IgM ELISA serve only as an aid to diagnosis and should be interpreted in relation to other clinical and diagnostic findings.
3. IgM responses may vary in different individuals. It has been reported that 10-30 % of infants may fail to develop IgM antibody responses despite congenital CMV infection. Furthermore, up to 27 % of adults with primary CMV infection may not demonstrate an IgM response. Thus, the absence of CMV-specific IgM does not necessarily exclude the possibility of CMV infection.
4. The presence or absence of CMV IgG or IgM in pregnant women is of limited value in predicting congenital CMV infection. However, the presence of specific IgM in the circulation of the newborn is indicative of infection. Since serum samples obtained too early in infection may not contain detectable IgM antibody, a subsequent sample should be obtained 7 to 14 days later and test. In the case of cord blood, care should be taken to avoid contamination by maternal blood, and it is prudent to confirm positive IgM antibody results by testing a follow-up specimen from the newborn.

#### **RELATED READING MATERIALS**

Voter, A., J.E. Bidwell, et al. Manual of clinical immunology\_ Chapter 69. Rose, N. and Friedman, H. eds. Am. Soc. Microbiol. **p.506, 1985.**

Cremer, N.E. Antibodies in serodiagnosis of viral infection. p. 73. In Lennett E.H. ed. Laboratory diagnosis of viral infection. Merck Dekker, Inc., New York, 1985.

Starr, S.E. and H.M. Friedman. "Human CMV." Chapter 65. In Manual of Clin. Microbiol., 4th ed., Lennett, E.H. et al ed. Am. Soc. Microbiol. pp. 771-719, 1985

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